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[^{18}F]Ethenesulfonyl fluoride as a novel radiofluoride relay reagent

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Abstract: Fluorine-18 is the most utilized radioisotope in Positron Emission Tomography (PET), but the wide application of fluorine-18 radiopharmaceuticals is hindered by its challenging labelling conditions. As such, many potentially important radiotracers remain underutilized. Herein, we describe the use of [^{18}F]ethenesulfonyl fluoride (ESF) as a novel radiofluoride relay reagent that allows radiofluorination reactions to be performed in minimally equipped satellite nuclear medicine centres. [^{18}F]ESF has a simple and reliable production route and can be stored on inert cartridges. The cartridges can then be shipped remotely and the trapped [^{18}F]ESF can be liberated by simple solvent elution. We have tested 18 radiolabelling precursors, inclusive of model and clinically used structures, and most precursors have demonstrated comparable radiofluorination efficiencies to those obtained using a conventionally dried [^{18}F]fluoride source.

Development of novel and efficient methods for incorporating fluorine-18 radioisotope into pharmaceutically relevant structures is of paramount importance for guaranteeing a wide access to PET tracers.[1-2] The traditional approach of nucleophilic substitution uses dried [^{18}F]fluoride and can only be performed in few centres with the appropriate level of equipment and expertise (e.g. cyclotron, synthesizers and bulky hot cells).[3] On the other hand, in most nuclear medicine departments where ^{99m}Tc is used, "shake & bake" labelling reactions are performed using minimal equipment,[4] thus providing access to a wide array of ^{99m}Tc radiotracers.[5] Similar protocols can also be applied to other radiometal labellings (e.g. ^{68}Ga, ^{64}Cu), but such simplicity has not yet been achieved for C-^{18}F labellings.[6-8]

To achieve such an important target, we focused on fluoride relay reagents,[9] where reactive fluoride species can be released following simple chemical or physical interaction. This interesting concept was reported recently by Pees, *et al*.[10] using [^{18}F]triflyl fluoride, and previously by DeGrado's group[11] using [^{18}F]acetyl fluoride. Both reagents were produced as gases and used immediately in their respective radiofluorination reactions. DeGrado's group also reported the feasibility of trapping [^{18}F]acetyl fluoride in a cartridge but limited information was given and the reaction scope was relatively narrow. While these approaches may potentially represent simpler ways to activate [^{18}F]fluoride (i.e. instead of azeotropic drying), it is unclear whether such reagents could be shipped conveniently for off-site use on a wider range of precursors.

Thereafter, [^{18}F]ethenesulfonyl fluoride (ESF) started to attract our attention. [^{18}F]ESF containing structures were recently reported to release [^{18}F]fluoride and therefore were not useful as PET tracers. However, ESF is liquid at room temperature, and has facile synthesis, simple purification and the feasibility to be trapped in a cartridge.[12] Therefore, [^{18}F]ESF might qualify as an optimal radiofluoride relay reagent with the capability of remote shipping. In this paper we test the application of [^{18}F]ESF to the radiofluorination of a variety of precursors, and compare the radiochemical yields (RCYs) to those using conventionally dried [^{18}F]fluoride method.

The synthesis of [^{18}F]ESF was performed in a microfluidic system using 2,4,6-trichlorophenylethenesulfonate and non-dried tetraethylammonium bicarbonate (TEAB)/[^{18}F]fluoride complex, and the produced [^{18}F]ESF was distilled onto a Silica-plus cartridge (**Figure 1** & ESI).[12] We usually produced 0.5–2.5 GBq of [^{18}F]ESF with an average RCY of 57±11% (n=24) and radiochemical purity (RP) >95%. The cartridge was then transported to the site of usage and eluted with chosen solvent. Stability was assessed by measuring the RP of the [^{18}F]ESF eluted from a dedicated cartridge after 4 h and the value remained unchanged. Alternatively, several Silica-light cartridges could be used to distribute the radioactivity over these supports, that could then be eluted with different solvents, providing further experimental flexibility. We believe the trapping of [^{18}F]ESF onto the Si matrix is mediated by non-covalent interactions involving the double bound, therefore making [^{18}F]ESF the smallest sulfonyl fluoride species with useful trapping features.[13] The scale of activity we used was intended to mimic levels that could be employed by a clinical radiopharmacy to obtain few doses of a particular ^{18}F radiopharmaceutical, or could be easily integrated in existing minimalistic systems already available to produce ^{99m}Tc, ^{68}Ga or other radiometal-based radiopharmaceuticals.[14-16] As a limit to this approach, the use of low activity did not allow to detect any UV signal for molar activity calculations; however, an estimation of molar activity obtained using no carrier added [^{18}F]ESF can be found in previous literature.[12]

In this work, our scope was to assess the feasibility of using [^{18}F]ESF on radiofluorinating a wide variety of precursors. Therefore, we did not optimize the RCY for each precursor, but instead looked for the impact of experimental conditions on RCYs. It is worth noticing that dedicated optimization studies would be needed for each precursor to obtain the best RCY.

Twelve precursors (**Figure 1**) were initially chosen to test the radiofluorination reactions with [^{18}F]ESF, of which **1-5** are model aromatic compounds, while **6-12** represent a set of commonly used radiotracer precursors for PET imaging. In a typical test, [^{18}F]ESF was eluted from the cartridge to obtain a stock solution in CH_3CN with a radioactivity concentration of 100–250 MBq/mL. An aliquot (200 µL, 20–50 Mbq) of this radiolabelling solution was then added to 800 µL of a premade solution or suspension of the

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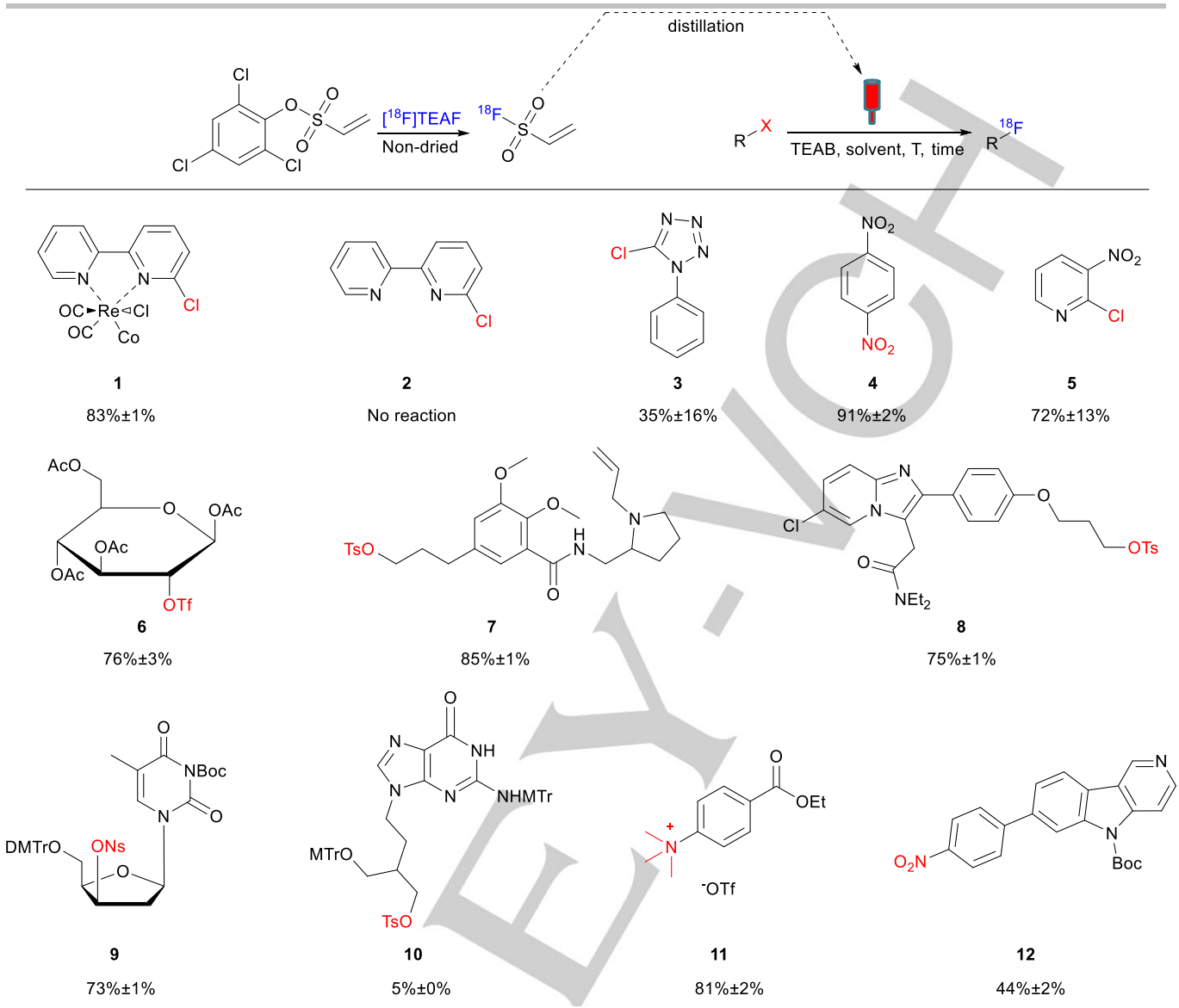

Figure 1 Tested precursors and best RCYs obtained, fluorination sites in red. General procedure: 200 µL [^{18}F]ESF in CH_3CN was added to 800 µL of precursor and TEAB in solvent and heated for 15 min. Conditions: **3**, **5-9**, and **11** (2mg/mL in CH_3CN, T=100°C), **10** (2 mg/mL in DMSO, T=100°C), **1**, **4** and **12** (1 mg/mL in DMSO, T=130°C).

desired precursor inside a single-use 4 mL glass vial. A suitable amount of TEAB was also added to release and activate the [^{18}F]fluoride. Alternatively, it is also possible to elute each cartridge directly with chosen solvent into a premade vial containing the dry precursors and TEAB. The reaction mixture was then magnetically stirred and heated using standard laboratory equipment. Each experiment was typically repeated three times and the RCY was calculated by analysing the reaction mixture with HPLC and correcting for the efficiency of radioactivity recovery of the liquid from the reaction vial (RCY = RCY_{HPLC} × $\%_{Recovery}$, see ESI). This correction, together with the use of a monolithic HPLC column,[17] allowed us to account for potential activity lost in the apparatus, therefore better estimating real-world production yields. As for the RCYs of **1** and **12**, we combined the RCYs for fluorination and for the radioproduct resulting from, respectively, dissociation of Re (unpublished work) and Boc deprotection.[18-19] A two-tail unpaired two-sample *t*-test was used to calculate the statistical significance between different sets of reactions. Precursor **2** was subjected to all the reaction sets, but no product was observed in any conditions.

Impact of TEAB was tested by varying its concentration from 0.01 to 0.50 mg/mL using a fixed amount of **11** (1 mg/mL). We found that TEAB was essential for the reaction, both acting as a base and a phase transfer catalyst to convert [^{18}F[ESF into reactive species [^{18}F]TEAF. In the absence of TEAB, Michael addition reaction could happen on free amines, as for substrate **12** at high temperatures; however, we verified that post-addition of TEAB to the same mixture successfully results in radiofluorination. The use of different bases (i.e. trimethylamine and $KHCO_3$) did not result in any radiofluorination. Good RCYs of 66% were already obtained using 0.05 mg/mL of TEAB; however, in order to simplify the operative parameters and to account for potential differences in precursor reactivity, we chose 0.5 mg/mL for the whole set of precursors. Such amount could be, in some cases, too basic for

some precursors and would need to be tuned appropriately in dedicated optimization studies.

Reaction time was next evaluated on a subset of precursors and RCY_{HPLC} were recorded at different time points (5, 10, 15 and 30 min, see ESI), sampling the reaction mixture each time after quenching *via* ice cooling. For most of the precursors, longer reaction times led to improved RCY_{HPLC}; however, such improvement would not always generate more final product due to radioactivity decay. In addition, ice cooling was essential to minimize the chances of producing hazardous radioactive volatiles; however, this operation did not warrant reaching the target temperature for all the duration of the time point tested.[20] We deemed that a reaction time of 15 min would be utilizable for obtaining acceptable radioactive incorporation with most of the precursors.

The effect of reaction temperature was tested on all the precursors under 3 conditions: 70, 100 and 130 °C (see ESI). Most precursors had very low RCYs (<10%) at 70 °C, while the precursors for [^{18}F]FDG and [^{18}F]fallypride (**6** and **7**) had RCYs >30%. RCYs were always statistically improved from 70 to 100 °C, but when reaching 130 °C, **5**-**9** and **11** provided lower RCYs. This is probably due to the decomposition of precursors at such higher temperature; in addition, we employed DMSO for all the reactions at 130 °C, even for the precursors which we previously investigated in CH_3CN (**3-9** and **11**), and such change could have also contributed to different RCYs.

Lastly, we studied the effect of precursor amount using concentrations of 0.5, 1 or 2 mg/mL, while the TEAB amount was kept at half of concentrations of the precursors (see ESI). The variation of RCYs due to this parameter was highly dependent on the precursors tested, with **6, 7, 9, 10** and **12** being the only ones showing statistically significant increases in RCYs when rising both from 0.5 to 1 mg/mL and from 1 to 2 mg/mL. The highest RCYs obtained and relative reaction conditions for all precursors after testing are summarised in **Figure 1**.

Bigger scale reactions employing ~100 MBq were also conducted on **7-9**, **11** and **12**. For **7** and **8**, the reaction mixture was passed through an alumina column and RCYs of 32% and 57% were obtained. For **9**, **11**, and **12**, radiofluorination did not provide the final product as additional steps were required to synthesize the target molecule. For example, **11** required two extra steps of a hydrolysis and a conjugation reaction to give the final product, [^{18}F]SFB, in 52% RCY (see ESI). We have also successfully tested acid hydrolysis for **9** and high-temperature deprotection for **12**, which further indicated that [^{18}F]ESF conditions do not interfere with subsequent additional reactions.

Comparative radiolabelling reactions using traditionally dried [^{18}F]tetraethylammonium fluoride (TEAF) were conducted using a homogenous set of conditions (same solvent, tetraethylammonium cation, precursor at 1 mg/mL, reaction time of 15 min, T=100 °C, see ESI). As shown in **Figure 2**, the RCYs using [^{18}F]ESF method were comparable to the use of traditionally dried [^{18}F]fluoride complex. In particular, the statistical test indicated that 6 precursors showed insignificant difference in RCYs, while for 4 precursors (**4**, **5**, **10** and **12**), the [^{18}F]ESF method provided higher RCYs; in the case of **3**, the traditional dried [^{18}F]TEAF provided a higher RCY. It was also observed that the standard deviations for these radiofluorinations were generally lower when using [^{18}F]ESF, which could be linked to the consistent quality of this labelling reagent compared to the [^{18}F]TEAF coming from a drying process that, even if automated, might introduce varying grades of moisture or other contaminants on each day it was performed, or due to the dissolution of the dried reagent back into solution.

We then moved our interest towards the use of [^{18}F]ESF for radiolabelling emerging precursors with novel leaving groups (i.e.

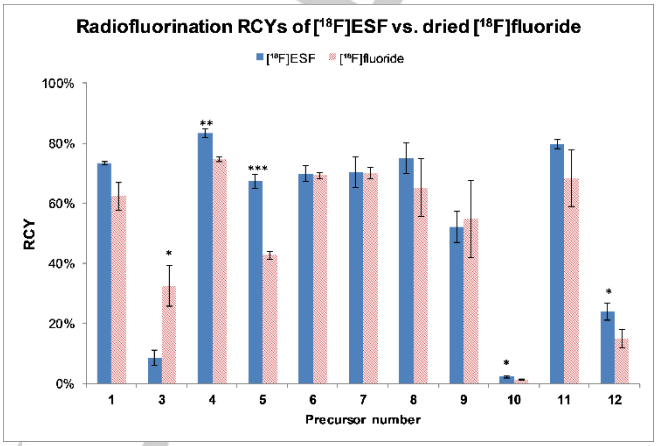

Figure 2 Comparative fluorination RCYs between [^{18}F]ESF and traditionally dried [^{18}F]TEAF at precursor concentration of 1 mg/mL, reaction time of 15 min and T=100 °C (n = 3), Whereas indicated, statistical significance was calculated by a two-tail unpaired two sample t-test (* p<0.05, ** p<0.005, ***p<0.0005).

boronic acids and iodonium ylides, **Figure 3**).[21-23] For boronic acid reactions, we started using 800 µL of 1 mg/mL of **13** in dimethylacetamide (DMA) added with 5 eq of $Cu(OTf)_2$, 125 eq of pyridine and 0.5 mg TEAB, and reacted with 200 µL of [^{18}F]ESF in CH_3CN at 110 °C for 20 min. Under these conditions, we obtained RCYs significantly lower than the ones reported in literature.[21] Given the potential impact of basic conditions on this type of reaction, we investigated decreasing the TEAB amount and realized that radiofluorination occurred even in absence of TEAB, albeit with the same low RCYs (see ESI). The effect of solvent was next tested, and we found that higher RCYs were obtained in absence of CH_3CN (i.e. in 100% DMA or DMF). In a last attempt to further improve RCYs, the effects of $Cu(OTf)_2$, pyridine and precursor concentration were investigated. We found a slightly increased RCY when 300 eq of pyridine were used. However, increasing the amount of $Cu(OTf)_2$ or decreasing amount of precursor did not provide any improvement. We therefore proceeded with the testing on all the precursors by reacting DMF eluted [^{18}F]ESF with 1 mg/mL precursor solutions in DMF, with 5 eq of $Cu(OTf)_2$, 125 eq of pyridine and without TEAB, heated at 110°C for 20 min. The RCYs obtained were reported in **Figure 3** and were compared to equivalent reactions performed using traditionally dried [^{18}F]TEAF. For all the reported precursors, the RCYs obtained using [^{18}F]ESF were lower than the ones obtained using [^{18}F]TEAF. However, we were surprised that this latter yield was drastically higher (18% in average) than equivalent literature values. This fact might be due to the use of [^{18}F]TEAF instead of $K_{2.2.2}$-based complexes reported previously, to different automated drying sequences or to other currently uncontrolled parameters. This could suggest that a deeper

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understanding of the crucial conditions for such Cu-mediated radiofluorinations is still needed.[24]

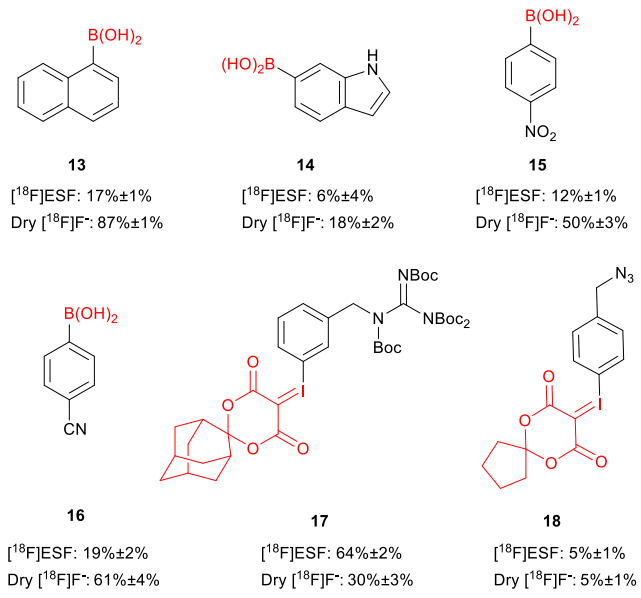

Figure 3 RCYs of novel types of precursors using [^{18}F]ESF and dried [^{18}F]TEAF under comparable conditions, fluorination sites are shown in red.

Radiofluorination reactions employing iodonium ylides were performed at 120 °C for 15 min using [^{18}F]ESF in CH_3CN and **17** and **18** in toluene and DMF respectively (see ESI). For these substrates, RCY of **17** (64±2%) was higher than the literature value (46%)[23] while RCY of **18** (5±1%) was lower than the literature value (25%).[25] This fact was not surprising, as iodonium reactivity can vary on each batch of precursor. In our setting, RCYs using [^{18}F]ESF were similar or better than using dried [^{18}F]TEAF.

In conclusion, we have demonstrated that [^{18}F]ESF is a simple to use radiofluoride relay reagent, providing RCYs comparable to dried [^{18}F]TEAF in most cases, but greatly reducing the reaction equipment needed, in the simplest case to a heating/stirring aluminium block and single-use vials. Currently, fluorine-18 is shipped either in an aqueous fluoride solution or inside an anion-exchange cartridge. Unlike [^{18}F]ESF cartridges, these forms would require additional drying steps, and cannot be used for radiofluorination in minimally equipped hospital or academia settings. As of now, in our simplified labelling approach, chromatographic purification and formulation is still needed to obtain the desired product. However, we believe that the ease introduced by [^{18}F]ESF fluorination process would justify the investigation into novel kit- or cartridge-based solutions. If such scenario will be realized, the miniaturization and compactification of quality control tests would provide the final tile to achieve an unprecedented flexibility of access to ^{18}F radiopharmaceuticals, thus providing the most personalized diagnoses possible.

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Financial supports from Monash University (MGS and MIPRS) and The Australian Institute of Nuclear Science and Engineering (AINSE PGRA 12074) are gratefully acknowledged. We also thank the ANSTO cyclotron team for providing [^{18}F]fluoride, and Dr. Tien Pham for his useful comments on manuscript editing.

Keywords: fluorine-18 • sulfonyl fluoride • radiofluorination • PET • ESF

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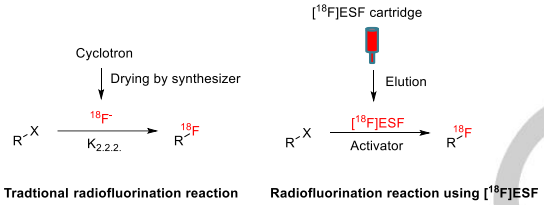
[^{18}F]ESF can be trapped into inert cartridges and released to provide a ready-to-use labelling solution, reducing the radiochemistry equipment needs.



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